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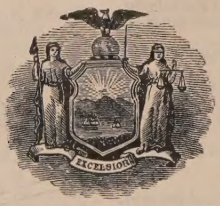
METHODS OF DETERMINING THE NUMBER OF MICRO-ORGANISMS IN TOMATO PRODUCTS

C. A. DARLING



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METHODS OF DETERMINING THE NUMBER OF MICRO-ORGANISMS IN TOMATO PRODUCTS

C. A. DARLING*

SUMMARY

Thirty samples of tomato pulp, containing varying amounts of decayed material, were prepared. The fresh pulp was examined by different methods to determine the number of micro-organisms present. After the examination, the pulp was sterilized by heating in a stoppered flask for one hour in running steam. A second examination was then made. This processed or sterilized pulp was concentrated and made into catsup, after which it was again examined and the number of micro-organisms determined.

The plate culture method was employed on the fresh pulp only; while the liquid-mount method, as described by Howard, and the dried-and-stained-smear method were used on the fresh pulp, processed pulp and catsup. This made a total of seven counts from each sample. Estimates were made of the amount of mold, the number of yeast cells and mold spores, the number of rod-shaped bacteria, and the total number of bacteria present.

After the first part of the work was completed a broth culture of *Bacillus subtilis* was added in definite proportions to each of three samples of fresh pulp, and examinations were made by the three methods to determine the number and the relative proportions of these bacteria which could be recovered. This work was repeated with a broth culture of *Bacterium coli*, and an additional analysis made after the samples had been sterilized and concentrated.

The results of the various analyses indicate that very large errors are found in the estimates of the number of micro-organisms, regardless of the method employed. Because of the heterogeneous character of the material, neither the agar plate nor the microscopic counts obtained appear to be as accurate as those secured from more homogeneous substances, such as milk. The organisms often remain attached to tomato particles and so are not uniformly distributed thru the pulp.

*Special agent of the New York Agricultural Experiment Station, cooperating with the Division of Biology and Agriculture of the National Research Council, Washington, D.C.

Counts of rod-shaped bacteria from duplicate samples of inoculated, unprocessed liquid and unstained material showed some variation among themselves and showed very wide variations from similar counts made from dried and stained mounts. The wide discrepancies in the counts from the fresh material seemed to be due largely to the difficulty of distinguishing between bacteria and cell debris in the unstained material.

Bacterial counts from uninoculated samples made from dried and stained mounts were almost invariably much larger when made from fresh material than when made from processed material, and counts from material subjected to prolonged processing with concentration showed still lower numbers. This effect is due to the destruction or loss of staining power of the bacteria in processed material and causes counts of this type to be very unsatisfactory. The effect noted is similar to, but more marked, than the effect produced by pasteurization on microscopic counts from dried and stained preparations of milk.

When counts were made carefully by the liquid-mount method, concentration caused an increase in the number of fragments of mold hyphæ, yeasts, spores, and bacteria per unit volume. While there were very wide individual variations, the average increase for 21 miscellaneous samples was approximately 100 per cent when the material was concentrated to one-half its original volume.

The number of any one type of micro-organism in a given sample, as determined by the methods employed, does not bear a very close relationship to the amount of decay in the original stock. While mold hyphæ, yeasts, spores, or bacteria in excess of the standards set by the United States Department of Agriculture Bureau of Chemistry were found in all material containing more than 4 to 7 per cent of decayed stock, it was impossible to tell the *relative amount* of decayed material present from the Howard counts. Samples known to contain only a small amount of unfit material sometimes showed mold hyphæ more than twice as abundant as samples known to contain as much as 44 per cent of decayed stock.

The accuracy of the counts by the Howard technic will undoubtedly be somewhat increased by the adoption in 1921 by the Association of Official Agricultural Chemists of modifications in this technic; but even with these improvements it cannot be regarded as a really satisfactory analytical method for use in control work. Perhaps the Howard technic can be still further improved so as to make it some-

what more satisfactory than it is at present. A more promising method of establishing a satisfactory control over the quality of this food product, however, seems to be thru the greater use of factory inspection at the time the catsup is made, supplemented by a laboratory examination of samples of fresh pulp in dried and stained mounts similar to those that are found satisfactory for controlling the quality of milk before pasteurization. The counts from such preparations seem to be more nearly accurate than those from the liquid mounts. Also, the preparations can be easily made and can be preserved for future reference in case any question arises later concerning the quality of the pulp used in manufacture. A control of this type would involve cooperation between federal and state officials.

INTRODUCTION

The number of micro-organisms in certain types of food has assumed such importance in the past few years with reference to public health that a consideration of the accuracy of the methods for determining the number of organisms present is of general interest. The public water supplies and public milk supplies have been given the most attention by sanitary officials in recent years. In the case of water less attention is given to the number of micro-organisms present than to their source, sewage contamination being considered a sufficient cause for condemnation of such water. In milk more attention is given to the number of micro-organisms present in a given quantity than to the kinds or sources of these organisms. This is due to the fact that it is difficult to detect the pathogenic and other undesirable kinds of bacteria which may be found in milk; and, also, because the presence of a large number of bacteria in milk indicates either old milk or improper and often unsanitary methods of handling. These facts have become so thoroly established that local boards of health in many cities of the United States require that milk offered for sale shall conform to certain bacterial count standards.

More recently, attention has been directed to the sanitary condition of other foods, including oysters, dried eggs, chopped meats, and various fruit and vegetable products. The Federal Food and Drug Act makes it illegal to ship from one state to another any food products made from putrid, filthy, or decomposed materials. The enforcement of this act necessitates either a very close factory

inspection at the time of manufacture of such foods, or a method of examination of the products after manufacture. For legal reasons the latter method has been the one generally found most effective by the federal authorities.

Without considering the various phases of the enforcement of this act it may be stated that the Bureau of Chemistry of the United States Department of Agriculture was given the work of detecting poor and adulterated foods. It was early found by the Bureau that among foods sometimes adulterated by food packers and canners were tomato pulp, puree, and catsup. This adulteration, in the meaning of the law, consisted in using poorly sorted tomatoes and wastes from the canning of tomatoes, including skins, cores, and trimmings which were often allowed to stand and frequently became fermented or even molded before being made into pulp or catsup. In view of these facts it was necessary to develop some method by means of which it would be possible to detect in the finished product the general condition of the materials from which the product was made.

Since decay in fruits is caused by micro-organisms including bacteria, yeasts, and molds, it is evident that the number of those micro-organisms present in the finished product may bear some relation to the character of the materials from which the product was made. In view of the fact that pulp and catsup are sterilized by heating and are usually placed in sterile, sealed containers, the culture method of determining the number of bacteria and other micro-organisms present in the food cannot be used as in the case of a sanitary analysis of water and milk. No chemical method has been developed to detect poor stock in the finished products, because of natural variations in composition which exist in these products. It remained for Howard of the Bureau of Chemistry to develop a method for examining such products. This method consists in examining portions of the product microscopically and counting the number of micro-organisms present in a given amount. The method is given in detail in United States Department of Agriculture Bulletin No. 581. By using this method rather extensivly both in the factories and in the laboratory, Howard and Stephenson (1)[1] have concluded that by good sanitary practice the so-called "mold count" may be kept down to 25 per cent or less of the microscopic fields, the yeast and spore count to 25 or less per 1/60 cmm., and the count of rod-shaped bacteria to 25 millions or less per cc.

[1]Figures in parenthesis refer to the Bibliography, page 55.

In a recent article Bitting (2) has called attention to the fact that of more than 9,000 Notices of Judgment of cases involving foods and drugs under the Federal Food and Drug Act over 800 have been upon tomatoes and tomato products. Some of these cases were for misbranding, but the majority were for adulteration in the sense of being made from decomposed stock. Bitting feels that much injustice is being done the tomato products industry because of the inaccurate results secured by the use of the Howard method of examination.

Many criticisms have been directed against Howard's method of examination so that when funds were offered to the Society of American Bacteriologists, the Committee on Research of the Society recommended that they be accepted and that the investigation be made under the auspices of the National Research Council. The funds were assigned by the Council to be used under the direction of Dr. R. S. Breed at the New York Agricultural Experiment Station. The present investigation was begun in August, 1920, and has been carried out by the author during the summer months at Geneva and during the winter in connection with his work as professor of biology at Allegheny College

GENERAL PLAN OF WORK

The procedure followed was to prepare pulp in the laboratory from tomatoes containing known amounts of decay and to determine the number of micro-organisms present in the fresh pulp by three different methods, *viz.*, the plate culture method, the dried-and-stained-smear method, and the Howard method. The pulp was then processed by boiling and tubed in sterile tubes. After several weeks this pulp was re-examined by the dried-and-stained-smear method and by the liquid-mount method, as used by Howard. After this examination the pulp was made into catsup by adding spices, sugar, salt, and vinegar, and concentrating to approximately one-half the volume. The catsup was later examined by the two methods used in the case of the processed pulp. This made a total of seven analyses from each sample.

The second part of the work consisted in adding to three samples of fresh pulp a broth culture of a bacterium so that the three cultures contained definite and known proportions of the broth culture. The inoculated samples, as well as the uninoculated fresh pulp, were tested for the number of micro-organisms present by the three methods

noted above. After this examination the samples were sterilized, concentrated by boiling, and placed in sterile tubes. After several weeks the samples were re-examined by the two latter methods.

EXPERIMENTAL RESULTS

EXAMINATION OF SAMPLES WITH KNOWN AMOUNTS OF DECAYED MATERIAL

Preparation of pulp.—Fresh tomatoes, some of which were partially decayed, were selected from the field. The decay was sometimes the black rot of the blossom end, but it was more frequently a soft rot around worm-holes or cracks in the tomato. In some cases the tomatoes were used as soon as brought from the field, while at other times they were allowed to stand in the laboratory for a day or more before being used. About 900 grams of tomatoes were used for each sample. The tomatoes were thoroly washed in tap water and the stems removed, no attempt being made to wash out the decayed portions in case such were present. The whole tomatoes were weighed and then the decayed parts were cut out and weighed and the percentage of decay computed. Only such portions as would generally be considered unfit for human consumption as a raw product were regarded as decayed. The tomatoes were then sliced, ground thru a food chopper, and brushed thru a sieve so that the pulp was practically of the same degree of fineness as that made in factories. Each sample prepared in this manner, was subjected to examination as soon as made.

Plate cultures.—The samples of pulp were diluted 10,000, 100,000, and 1,000,000 times with sterile water. Five plates were made of each dilution, in most cases on plain nutrient agar. The plates were incubated at 37° C. for 48 hours, the total number of colonies counted on each plate, and the average computed. Eight different types of nutrient agar were used on the first ten samples tested. These media varied as to acidity, the presence or absence of dextrose or lactose and as to the presence or absence of tomato juice. With hardly an exception, the medium which gave the highest counts was the standard nutrient agar, consisting of 1.5 per cent agar, 0.3 per cent Liebig's beef extract, and 0.5 per cent Difco peptone, with a pH value of 7.0.

Dried-and-stained-smear mount.—The general method used was the direct microscopic method as recommended in the Standard Methods for the Examination of Milk (3). The pulp or catsup was

diluted with sterile water, usually two or eight times, depending upon the concentration of the material. The dilution was made according to Howard's method as follows: After thoro shaking, 10 cc. of the material were placed in a graduated cylinder and the required amount of water added. The mixture was then thoroly shaken and poured into a beaker; 0.01 cc. of the material was taken with a pipette graduated to 0.01 cc. and, with the aid of a needle, smeared as evenly as possible on a glass slide over 1 sq.cm. of surface. The water was evaporated quickly over a flame because with slow evaporation there was a tendency for the larger particles to be pulled toward the center of the drop. After being dried, a few drops of ethyl alcohol were placed on the slide for one or two minutes to insure fixation of material on the slide. The smear thus made was stained for about one minute in a fresh, saturated, aqueous solution of methylene blue. The smear was then washed in water until the excess stain was removed, and then dried. Four smears were made from each sample tested. Different types of stains and different methods of staining were tried, but methylene blue gave the best results.

In counting the number of micro-organisms a Spencer 1.5 mm. oil immersion objective was used with an x 10 eyepiece. An eyepiece disc divided into quadrants was used for convenience in counting, also a mechanical hand tally. The area of the microscopic field within the circle of the eyepiece was 0.007854 sq.mm., the number of microscopic fields in one square centimeter being 12,732. In the first series of fresh and of processed pulp 100 microscopic fields from the four smears were examined. In order to estimate the number of micro-organisms in a cubic centimeter of the material, the actual number counted was multiplied by 12,732 times the dilution, which was usually three. The factor used in multiplying was, therefore, 38,196, as compared to 10,000, 100,000, or 1,000,000 in the plate method. In the catsup only 25 fields were counted for bacteria and 100 fields for hyphal fragments and for yeasts and spores. The draw tube was so adjusted that the factor was 50,000 times the dilution. This limited number of fields was counted so that the factor for multiplication would be the same as in the liquid mount. Counts were made of fragments of hyphæ, yeasts and spores, total bacteria, and rod-shaped bacteria. Fragments of hyphæ were considered to be such in case the length of the fragment was more than one and one-half times its diameter. The yeast and spore count included the spores and conidia of molds not included in the hyphal fragment count, each

yeast cell being counted even tho united in filaments. As a rule the particles of cellular debris were distinguishable from the bacteria by being more irregular in form and less densely stained. The bacteria counted as rods were those in which the length was more than one and one-half times the diameter.

Liquid mount or Howard method.—The method as detailed by Howard and Stephenson (1) was followed, the only modification being that in counting bacteria in the fresh and processed pulp double the number of areas specified were examined. Spencer apochromatic objectives and compensating oculars were used. Counts were made of the total number of bacteria as well as of the rod-shaped bacteria, the two counts being made from the same mount. The bacteria counted as rods were more than one and one-half times as long as broad. In counting from the unstained mounts it was difficult to distinguish with certainty between bacteria and particles of pulp. In some cases there was sufficient difference in the refractive index to make it possible to recognize the bacteria, but in some material in which motile bacteria were present this difference could not be distinguished. The outline of the bacteria was usually regular but even this characteristic did not always permit the recognition of the bacteria with certainty.

In estimating the number of yeasts and spores, the number in 1 cc. was determined, so that the results obtained could be compared directly with those from the dried and stained mounts. Since 1/20 cmm. of solution was observed in the fresh and processed pulp, the multiplication factor was 20,000 times the dilution, or 60,000 as a rule. In counting the bacteria 1/80 cmm. was observed. The factor used in transforming to the per cc. basis, therefore, was 80,000 times the dilution, or 240,000 as a rule, in comparison with 38,196 for the dried and stained mount, and 10,000, 100,000, or 1,000,000 for the plate count. It should be noted that in making both the dried and stained mount and the liquid mount the material was taken from the same diluted sample, the dilution being made according to the Howard method. In the examination of catsup, ½ cmm. of material was examined in making the yeast and spore count and 1/50 cmm. in making the bacterial counts. This is a larger amount than is specified by Howard and reduced the multiplication factor in the first case to 2,000 times the dilution and, in the second case, to 50,000 times the dilution.

Computations of the results were done in most cases with a computing machine. In recording the results (Tables 1 to 8) not more than three significant figures have been reported.

TABLE 1.—AMOUNT OF MOLD IN 30 SAMPLES OF FRESH PULP, PROCESSED PULP, AND CATSUP, ESTIMATED BY DIFFERENT METHODS.

SAMPLE NUMBER	PERCENTAGE OF ROT	PERCENTAGE OF MICROSCOPIC FIELDS SHOWING MOLD HYPHAE			STAINED MOUNT, NUMBER OF HYPHAL FRAGMENTS PER CC.			LIQUID MOUNT, NO. HYPHAL FRAGMENTS PER CC.
		Fresh pulp	Processed pulp	Catsup	Fresh pulp	Processed pulp	Catsup	Catsup
2a	0	2	2	0	38,000	0	0	0
3b	0	0	0	0	0	0	0	0
4a	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8b	0	0	0	0	0	0	0	0
13a	3.5	82	86	100	840,000	1,380,000	1,800,000	1,220,000
13b	4	30	16	42	229,000	153,000	225,000	240,000
14a	4	72	72	100	191,000	726,000	1,730,000	1,350,000
14b	4	28	22	54	38,000	38,000	75,000	323,000
11b	4.5	52	46	90	420,000	611,000	600,000	803,000
10b	5	22	12	16	229,000	229,000	225,000	120,000
1a	8	6	30	34	76,000	153,000	125,000	203,000
9b	8	60	40	46	344,000	38,000	225,000	323,000
10a	8	98	100	100	2,600,000	3,170,000	6,300,000	5,760,000
11a	9	66	90	98	955,000	1,410,000	900,000	1,310,000
8a	11	86	82	100	1,570,000	1,300,000	3,380,000	3,620,000
1b	16	46	54	82	38,000	115,000	75,000	795,000
31	17	4	14	22	802,000	458,000	400,000	120,000
2b	22	94	100	100	1,640,000	2,410,000	7,950,000	Indefinite
3a	30	98	100	100	1,800,000	1,600,000	7,500,000	Indefinite
9a	38	96	96	100	1,150,000	1,460,000	2,700,000	3,170,000
23	0	0			229,000			
21	7	16			1,830,000			
30	18	2			0			
24	20	82			115,000			
26	21	96			3,440,000			
27	44	32			917,000			
19	100	100			13,500,000			
20	100	98			917,000			
25	100	100			11,300,000			

TABLE 2.—PERCENTAGE OF MOLD FOUND IN NINE SAMPLES OF PROCESSED PULP AND CATSUP, AS COMPARED WITH FRESH PULP.
Computed from results given in Table 1.

SAMPLE NUMBER	LIQUID MOUNTS*			STAINED MOUNTS*		
	Fresh pulp	Processed pulp	Catsup	Fresh pulp	Processed pulp	Catsup
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
31	100	350.0	550.0	100	57.1	49.9
1a	100	500.0	566.7	100	200.0	163.6
10b	100	54.5	72.7	100	100.0	98.1
14b	100	78.6	192.9	100	100.0	196.3
13b	100	53.3	140.0	100	66.7	98.2
1b	100	117.4	178.3	100	300.0	196.3
11b	100	88.5	172.7	100	145.4	142.8
9b	100	66.7	76.7	100	11.1	65.4
11a	100	136.4	148.5	100	148.0	94.3

*Results from liquid mounts based on percentage of fields showing mold, those from stained mounts based on the number of hyphal fragments.

EXAMINATION OF HIGH GRADE PULP INOCULATED WITH ROD-SHAPED BACTERIA

In order to determine the accuracy of the results obtained in counting bacteria in tomato products, a definite amount of a broth culture of a known organism was added to some freshly made high grade pulp containing relatively few micro-organisms. Two such series of experiments were performed. In the first series a broth culture of *Bacillus subtilis* was used, and in the second series a culture of *Bacterium coli*.

Series 1.—In the first series, pulp was made from fresh, sound, well-ripened tomatoes, care being taken to keep the bacterial count as low as possible. To 500 cc. of this pulp 25 cc. of a 20-hour-old broth culture of *B. subtilis* were added and the two thoroly mixed by shaking. This mixture was designated as Sample As. To 200 cc. of Sample As were added 200 cc. of the original pulp, thus diluting the material one-half. The new dilution was designated as Sample Bs. To 200 cc. of Sample Bs were added 600 cc. of fresh pulp, thus diluting the original broth culture eight times. This dilution was designated as Sample Cs. The number of rod-shaped bacteria in the original pulp, as well as in each of the three samples, was estimated by the plate, the liquid-mount, and the dried-and-stained-mount methods. The details followed in making these estimates were the same as those used in the first part of this investigation except that 1 /50,000 cc. of diluted material was observed in both the liquid and in the dried and stained

TABLE 3.—NUMBER OF YEAST CELLS AND SPORES PER CC. IN 30 SAMPLES OF FRESH PULP, PROCESSED PULP, AND CATSUP, ESTIMATED BY DIFFERENT METHODS.

SAMPLE NUMBER	PERCENTAGE OF ROT	FRESH PULP		PROCESSED PULP		CATSUP			
		Liquid mount*	Stained mount	Liquid mount*	Stained mount	Liquid mount A*	Liquid mount B†	Percentage variation	Stained mount
2a	0	240,000	535,000	60,000	0	120,000	228,000	90.0	0
3b	0	30,000	1,380,000	150,000	191,000	960,000	1,320,000	37.5	300,000
4a	0	0	38,000	0	0	720,000	336,000	114.3	150,000
7	0	0	306,000	0	0	120,000	108,000	11.1	0
8b	0	0	153,000	30,000	0	240,000	168,000	42.9	0
13a	3.5	1,560,000	2,330,000	1,500,000	2,560,000	2,880,000	2,990,000	3.8	2,550,000
13b	4	420,000	688,000	300,000	267,000	1,440,000	1,740,000	20.8	375,000
14a	4	1,860,000	993,000	810,000	879,000	3,720,000	2,950,000	26.1	1,050,000
14b	4	180,000	267,000	120,000	267,000	240,000	492,000	105.0	225,000
11b	4.5	420,000	1,410,000	180,000	840,000	600,000	1,510,000	152.0	375,000
10b	5	60,000	2,640,000	150,000	1,830,000	1,560,000	1,610,000	3.2	2,030,000
1a	8	180,000	1,680,000	60,000	497,000	480,000	800,000	66.7	1,560,000
9b	8	480,000	1,030,000	30,000	191,000	120,000	516,000	330.0	375,000
10a	8	5,280,000	3,170,000	2,460,000	1,990,000	4,920,000	6,000,000	22.0	3,000,000
11a	9	1,680,000	2,330,000	1,050,000	2,860,000	1,560,000	1,960,000	25.6	1,050,000
8a	11	4,200,000	8,590,000	2,280,000	3,740,000	3,600,000	3,370,000	6.8	4,880,000
1b	16	420,000	382,000	420,000	153,000	240,000	192,000	25.0	150,000
31	17	180,000	3,320,000	360,000	573,000	0	352,000		1,000,000
2b	22	4,260,000	23,300,000	27,400,000	17,500,000	33,000,000	38,700,000	17.3	20,500,000
3a	30	7,200,000	13,400,000	10,500,000	8,600,000	10,400,000	11,500,000	10.6	13,700,000
9a	38	1,800,000	7,560,000	2,670,000	4,200,000	5,160,000	5,940,000	15.1	11,000,000
23	0	720,000	6,420,000						
21	7	4,500,000	10,500,000						
30	18	180,000	3,210,000						
24	20	180,000	4,010,000						
26	21	3,960,000	7,560,000						
27	44	3,960,000	15,600,000						
19	100	34,000,000	7,640,000						
20	100	4,320,000	2,750,000						
25	100	20,200,000	20,200,000						
Totals for first 21 samples		30,500,000	75,500,000	50,500,000	47,100,000	72,100,000	82,800,000		64,300,000

*Computed on counting 1/20 cmm.
†Computed on counting ⅓ cmm.

TABLE 4.—PERCENTAGE OF YEASTS AND SPORES FOUND IN 21 SAMPLES OF PROCESSED PULP AND CATSUP, AS COMPARED WITH FRESH PULP.

Computed from results given in Table 3.

SAMPLE NUMBER	LIQUID MOUNT				STAINED MOUNT		
	Fresh pulp	Processed pulp	Catsup, mount A	Catsup, mount B	Fresh pulp	Processed pulp	Catsup
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2a	100	25.0	50.0	95.0	100	0.0	0.0
3b	100	500.0	3200.0	4400.0	100	13.8	21.7
4a	100	100.0	?	?	100	0.0	394.0
7	100	100.0	?	?	100	0.0	0.0
8b	100	?	?	?	100	0.0	0.0
13a	100	96.2	185.0	192.0	100	110.0	109.0
13b	100	71.4	343.0	438.0	100	38.8	54.5
14a	100	43.6	200.0	159.0	100	88.5	106.0
14b	100	66.7	133.0	273.0	100	100.0	84.3
11b	100	42.9	143.0	360.0	100	59.6	26.6
10b	100	250.0	2600.0	2670.0	100	69.3	76.8
1a	100	36.7	267.0	444.0	100	29.6	92.8
9b	100	6.3	25.0	108.0	100	18.5	36.4
10a	100	46.6	93.2	114.0	100	62.8	94.6
11a	100	62.5	92.9	117.0	100	123.0	45.1
8a	100	54.3	85.7	80.2	100	43.5	56.8
1b	100	100.0	57.1	45.7	100	40.0	39.3
31	100	200.0	0.0	196.0	100	17.3	30.1
2b	100	643.0	775.0	909.0	100	75.1	88.0
3a	100	146.0	144.0	160.0	100	64.2	102.0
9a	100	148.0	287.0	330.0	100	55.6	146.0

TABLE 5.—NUMBER OF ROD-SHAPED BACTERIA PER CC. IN 30 SAMPLES OF FRESH PULP, PROCESSED PULP, AND CATSUP, ESTIMATED BY DIFFERENT METHODS.

SAMPLE NUMBER	PERCENTAGE OF ROT	FRESH PULP		PROCESSED PULP		CATSUP			
		Liquid mount	Stained mount	Liquid mount	Stained mount	Liquid mount A*	Liquid mount B†	Percentage variation	Stained mount
2a	0	2,160,000	6,650,000	13,000,000	879,000	64,300,000	71,100,000	10.6	4,800,000
3b	0	2,400,000	5,000,000	2,880,000	267,000	97,000,000	84,000,000	15.5	4,200,000
4a	0	1,440,000	3,170,000	6,480,000	1,410,000	64,300,000	66,900,000	4.0	4,800,000
7	0	2,880,000	2,560,000	3,600,000	1,260,000	36,500,000	47,400,000	29.9	1,500,000
8b	0	6,000,000	2,410,000	8,160,000	2,410,000	24,000,000	23,400,000	2.6	2,400,000
13a	3.5	24,700,000	78,900,000	92,400,000	48,500,000	68,200,000	78,000,000	14.4	25,200,000
13b	4	76,300,000	215,000,000	93,600,000	108,000,000	68,200,000	65,100,000	4.8	37,200,000
14a	4	80,400,000	162,000,000	63,600,000	50,700,000	89,300,000	103,000,000	15.3	51,600,000
14b	4	51,400,000	93,200,000	49,700,000	39,400,000	49,000,000	52,500,000	7.2	20,100,000
11b	4.5	69,100,000	185,000,000	59,800,000	119,000,000	73,000,000	72,300,000	2.2	32,100,000
10b	5	35,300,000	106,000,000	29,500,000	40,200,000	138,000,000	133,000,000	3.8	21,600,000
1a	8	25,700,000	37,400,000	13,700,000	6,610,000	134,000,000	120,000,000	11.7	8,750,000
9b	8	112,000,000	325,000,000	115,000,000	165,000,000	113,000,000	109,000,000	3.7	61,800,000
10a	8	67,400,000	171,000,000	96,700,000	82,200,000	136,000,000	141,000,000	3.7	78,600,000
11a	9	63,600,000	158,000,000	70,100,000	95,000,000	77,800,000	76,500,000	1.7	41,400,000
8a	11	87,100,000	140,000,000	35,500,000	68,900,000	83,500,000	94,200,000	12.8	31,200,000
1b	16	22,100,000	93,500,000	28,100,000	28,200,000	147,000,000	149,000,000	1.4	15,900,000
31	17	14,400,000	27,700,000	31,700,000	14,600,000	128,000,000	82,400,000	55.3	8,000,000
2b	22	90,200,000	393,000,000	119,000,000	126,000,000	322,000,000	327,000,000	1.6	80,100,000
3a	30	112,000,000	590,000,000	330,000,000	234,000,000	324,000,000	331,000,000	2.2	171,000,000
9a	38	148,000,000	533,000,000	149,000,000	184,000,000	206,000,000	276,000,000	34.0	125,000,000
23	0	13,600,000	15,000,000						
21	7	10,200,000	74,200,000						
30	18	22,400,000	54,700,000						
24	20	156,000,000	327,000,000						
26	21	146,000,000	230,000,000						
27	44	234,000,000	149,000,000						
19	100	57,600,000	78,900,000						
20	100	1,300,000,000	2,000,000,000						
25	100	136,000,000	499,000,000						
Totals for first 21 samples		1,100,000,000	3,330,000,000	1,410,000,000	1,420,000,000	2,440,000,000	2,500,000,000		827,000,000

*Computed on counting 5/800 cmm.

†Computed on counting 16/800 cmm.

TABLE 6.—PERCENTAGE OF ROD-SHAPED BACTERIA FOUND IN 21 SAMPLES OF PROCESSED PULP AND CATSUP AS COMPARED WITH FRESH PULP.

Computed from results given in Table 5.

SAMPLE NUMBER	LIQUID MOUNT				STAINED MOUNT		
	Fresh pulp	Processed pulp	Catsup, mount A	Catsup, mount B	Fresh pulp	Processed pulp	Catsup
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2a	100	602.0	2980.0	3290.0	100	13.2	72.2
3b	100	120.0	4040.0	3500.0	100	5.3	84.0
4a	100	450.0	4470.0	4650.0	100	44.5	151.0
7	100	125.0	1270.0	1610.0	100	49.2	58.6
8b	100	136.0	400.0	390.0	100	100.0	99.6
13a	100	374.0	276.0	316.0	100	61.5	31.9
13b	100	123.0	89.4	85.3	100	50.2	17.3
14a	100	79.1	111.0	128.0	100	31.3	31.9
14b	100	96.7	95.3	102.0	100	42.2	21.6
11b	100	86.5	107.0	105.0	100	64.3	17.4
10b	100	83.6	391.0	377.0	100	37.9	20.4
1a	100	53.3	521.0	467.0	100	17.7	23.4
9b	100	103.0	101.0	97.3	100	50.8	19.0
10a	100	143.0	202.0	209.0	100	48.1	46.0
11a	100	110.0	122.0	120.0	100	60.1	26.2
8a	100	40.8	95.9	108.0	100	49.2	22.3
1b	100	127.0	665.0	674.0	100	30.2	17.0
31	100	220.0	889.0	572.0	100	52.7	28.9
2b	100	132.0	357.0	363.0	100	32.1	20.4
3a	100	295.0	289.0	296.0	100	39.7	29.0
9a	100	100.0	138.0	185.0	100	34.5	23.5

TABLE 7.—TOTAL NUMBER OF BACTERIA PER CC. IN 30 SAMPLES OF FRESH PULP, PROCESSED PULP, AND CATSUP, ESTIMATED BY DIFFERENT METHODS.

SAMPLE NUMBER	PERCENTAGE OF ROT	FRESH PULP		PROCESSED PULP			CATSUP	
		Agar plate count	Liquid mount	Stained mount	Liquid mount	Stained mount	Liquid mount	Stained mount
2a	0	4,490,000	11,300,000	8,480,000	24,200,000	1,490,000	136,000,000	4,800,000
3b	0	240,000	11,300,000	7,260,000	8,160,000	344,000	219,000,000	4,500,000
4a	0	620,000	7,200,000	4,890,000	13,700,000	1,720,000	138,000,000	6,600,000
7	0	90,000	13,900,000	3,210,000	12,000,000	2,180,000	104,000,000	1,800,000
8b	0	610,000	12,700,000	3,170,000	16,800,000	3,250,000	45,000,000	3,000,000
13a	3.5	19,000,000	61,200,000	105,000,000	162,000,000	65,700,000	133,000,000	32,100,000
13b	4	99,000,000	142,000,000	254,000,000	168,000,000	123,000,000	119,000,000	38,400,000
14a	4	69,000,000	169,000,000	203,000,000	99,600,000	58,500,000	182,000,000	57,900,000
14b	4	51,000,000	106,000,000	113,000,000	80,400,000	47,600,000	92,100,000	23,400,000
11b	4.5	82,000,000	129,000,000	208,000,000	108,000,000	135,000,000	142,000,000	34,800,000
10b	5	45,000,000	71,000,000	129,000,000	63,100,000	48,500,000	184,000,000	24,900,000
1a	8	31,000,000	67,900,000	56,000,000	27,600,000	8,020,000	193,000,000	9,250,000
9b	8	218,000,000	204,000,000	373,000,000	202,000,000	186,000,000	187,000,000	69,600,000
10a	8	185,000,000	144,000,000	228,000,000	151,000,000	112,000,000	257,000,000	93,600,000
11a	9	203,000,000	120,000,000	192,000,000	133,000,000	117,000,000	122,000,000	45,600,000
8a	11	532,000,000	220,000,000	216,000,000	91,400,000	106,000,000	172,000,000	40,500,000
1b	16	81,000,000	73,200,000	116,000,000	56,600,000	35,200,000	222,000,000	16,800,000
31	17	8,030,000	40,300,000	37,000,000	61,200,000	19,500,000	127,000,000	11,200,000
2b	22	182,000,000	231,000,000	538,000,000	241,000,000	167,000,000	462,000,000	88,800,000
3a	30	298,000,000	259,000,000	711,000,000	447,000,000	272,000,000	449,000,000	193,000,000
9a	38	87,000,000	277,000,000	640,000,000	252,000,000	210,000,000	341,000,000	140,000,000
23	0	190,000	70,400,000	28,600,000				
21	7	45,000,000	52,500,000	166,000,000				
30	18	3,300,000	59,200,000	70,600,000				
24	20	422,000,000	413,000,000	599,000,000				
26	21	5,500,000	300,000,000	376,000,000				
27	44	210,000,000	614,000,000	239,000,000				
19	100	138,000,000	240,000,000	311,000,000				
20	100	2,440,000,000	2,820,000,000	3,750,000,000				
25	100	212,000,000	243,000,000	887,000,000				
Totals for first 21 samples		2,200,000,000	2,370,000,000	4,150,000,000	2,420,000,000	1,720,000,000	4,030,000,000	941,000,000

TABLE 8—PERCENTAGE OF BACTERIA FOUND IN 21 SAMPLES OF PROCESSED PULP AND CATSUP, AS COMPARED WITH FRESH PULP.

Computed from results given in Table 7.

SAMPLE NUMBER	LIQUID MOUNT			STAINED MOUNT		
	Fresh pulp	Processed pulp	Catsup	Fresh pulp	Processed pulp	Catsup
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2a	100	214.0	1200.0	100	17.6	56.6
3b	100	72.2	1940.0	100	4.7	62.0
4a	100	190.0	1920.0	100	35.2	135.0
7	100	86.3	748.0	100	67.9	56.1
8b	100	132.0	354.0	100	103.0	94.6
13a	100	265.0	217.0	100	62.6	30.6
13b	100	118.0	83.8	100	48.4	15.1
14a	100	58.9	107.0	100	28.8	28.5
14b	100	75.8	86.8	100	42.1	20.7
11b	100	83.7	110.0	100	64.9	16.7
10b	100	88.9	259.0	100	37.6	19.3
1a	100	40.6	284.0	100	14.3	16.5
9b	100	99.0	91.7	100	49.9	18.7
10a	100	105.0	178.0	100	49.1	41.1
11a	100	111.0	102.0	100	60.9	23.7
8a	100	41.5	78.2	100	49.1	18.8
1b	100	77.3	303.0	100	30.3	14.5
31	100	152.0	315.0	100	52.7	30.3
2b	100	104.0	200.0	100	31.0	16.5
3a	100	173.0	173.0	100	38.3	27.1
9a	100	91.0	123.0	100	32.8	21.9

mounts, the dilution being 1:8. The factor for multiplication, therefore, was 400,000.

The routine for examination was as follows: A portion from the original pulp and from each of the samples was taken, diluted, three dried mounts prepared, and an immediate examination made by the liquid mount method. As soon as the examination of the liquid mount was completed, a portion was taken from the original pulp and from each of the samples, diluted 10,000, 50,000, 500,000, or 1,000,000 times, and plated on nutrient agar. The samples and pulp were kept in stoppered flasks set in ice water in a refrigerator. The temperature of the material ranged from 3°C. to 5°C. during the period of examination. This routine procedure was followed until ten duplicate portions from Samples As, Bs, and Cs, and five duplicate portions of the original pulp had been examined by each of the three methods. The examinations were begun August 24 and completed August 28, except for the counting of the dried mounts, which was completed later. Triplicate agar plates were made of each sample, except Sample As, and the plates were incubated 48 hours at 37°C. and 48 hours at 25°C.

Tables 9 to 17, inclusive, show the results obtained. The ratios between the counts of *B. subtilis* made from Samples As, Bs, and Cs were determined after subtracting the total number of rod-shaped bacteria in the original pulp (Tables 9 and 10) from the number present in the inoculated samples (Tables 11 to 16). The estimated numbers and the ratios obtained are shown in Table 17.

TABLE 9.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH UNINOCULATED, HIGH GRADE PULP.

SAMPLE	NUMBER OF COLONIES ON PLATES, DILUTION 1:5,000			COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	9	3	4	26,700	+29.0
b′	4	1	1	13,300	-35.7
c′	5	20*	0	41,700	+101.0
d′	2	5	0	11,700	-43.5
e′	2	1	3	10,000	-51.7
Average				20,700†	

*Includes a cluster of 18.
†Only approximately correct as too great a dilution was used.

TABLE 10.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF THE FRESH UNINOCULATED PULP.

SAMPLE*	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	61,600,000	−23.3	3,200,000	−16.7
b	70,400,000	−12.4	4,400,000	+14.6
c	92,800,000	+15.4	2,800,000	−27.1
d	90,800,000	+12.9	3,200,000	−16.7
e	86,400,000	+ 7.5	5,600,000	+45.8
Averages	80,400,000		3,840,000	

*The dilutions for Sample a′, Table 9, were prepared at the same time as those for Sample a; those for Sample b′, Table 9, at the same time as those for Sample b, etc.

TABLE 11.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH PULP TO WHICH *B. subtilis* HAD BEEN ADDED (SAMPLE As).

SAMPLE	NUMBER OF COLONIES ON PLATES, DILUTION 1 : 10,000		COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2		
a′	72	92	820,000	−56.4
b′	294	254	2,740,000	+45.7
c′	203	229	2,160,000	+14.9
d′	30	33	315,000	−83.2
e′	260	347	3,040,000	+61.7
f′	532	233	3,830,000	+103.7
g′	28	23	255,000	−86.4
h′	218	272	2,450,000	+30.3
i′	137	spreader	1,370,000	−27.1
j′	179	spreader	1,790,000	−4.8
Average			1,880,000	

Note: The variation in number of colonies from duplicate plates was due, in part, to clusters of colonies.

TABLE 12.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF FRESH PULP TO WHICH *B. subtilis* HAD BEEN ADDED (SAMPLE As).

SAMPLE*	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	206,000,000	–2.8	25,200,000	–4.6
b	329,000,000	+55.2	29,600,000	+12.1
c	168,000,000	–20.7	22,800,000	–13.6
d	228,000,000	+7.5	16,400,000	–37.9
e	173,000,000	–18.4	30,800,000	+16.7
f	212,000,000	0.0	42,000,000	+59.1
g	184,000,000	–13.2	28,400,000	+7.6
h	198,000,000	–6.6	18,400,000	–30.3
i	196,000,000	–7.6	27,200,000	+3.0
j	228,000,000	+7.5	23,600,000	–10.6
Averages	212,000,000		26,400,000	

*The dilutions for Sample a′, Table 11, were prepared at the same time as those for Sample a; those for Sample b′, Table 11, at the same time as those for Sample b, etc.

TABLE 13.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH PULP TO WHICH *B. subtilis* HAD BEEN ADDED. (SAMPLE Bs).

SAMPLE	NUMBER OF COLONIES ON PLATES, DILUTION 1:10,000			COUNT PER CC.	PERCENTAGE VARIATION FROM THE AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	92	95	123	1,030,000	–26.7
b′	124	139	155	1,390,000	–1.4
c′	132	150	spreader	1,410,000	0.0
d′	178	190	spreader	1,840,000	+30.5
e′	101	120	123	1,150,000	–18.4
f′	112	147	127	1,290,000	+8.5
g′	153	148	156	1,520,000	+7.8
h′	210	138	108	1,520,000	+7.8
i′	155	145	143	1,480,000	+5.0
j′	138	150	162	1,500,000	+6.4
Average				1,410,000	

TABLE 14.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF FRESH PULP TO WHICH *B. subtilis* HAD BEEN ADDED. (SAMPLE Bs).

SAMPLE*	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	177,000,000	+1.1	7,000,000	-46.2
b	200,000,000	+14.3	4,000,000	-69.2
c	173,000,000	-1.2	13,200,000	+1.5
d	164,000,000	-5.7	14,800,000	+13.8
e	162,000,000	-7.4	11,600,000	-10.8
f	197,000,000	+12.6	11,200,000	-13.9
g	172,000,000	-1.9	20,400,000	+56.9
h	181,000,000	+3.4	20,800,000	+60.0
i	171,000,000	-2.3	10,800,000	-16.9
j	152,000,000	-13.4	16,400,000	+26.2
Averages	175,000,000		13,000,000	

*The dilutions for Sample a′, Table 13, were prepared at the same time as those for Sample a; those for Sample b′, Table 13, at the same time as those for Sample b, etc.

TABLE 15.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH PULP TO WHICH *B. subtilis* HAD BEEN ADDED. (SAMPLE Cs).

SAMPLE	NUMBER OF COLONIES ON PLATES, DILUTION 1:10,000			COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	44	69	59	573,000	-12.3
b′	28	129	119	920,000	+40.9
c′	41	121	47	697,000	+6.7
d′	83	124	73	933,000	+42.9
e′	54	47	125	760,000	+16.4
f′	24	39	16	263,000	-59.7
g′	63	55	58	587,000	-10.1
h′	166	49	43	860,000	+31.7
i′	49	38	34	403,000	-38.3
j′	52	50	58	533,000	-18.4
Average				653,000	

TABLE 16.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF FRESH PULP TO WHICH *B. subtilis* HAD BEEN ADDED. (SAMPLE Cs).

SAMPLE*	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	43,800,000	−58.3	11,800,000	+103.0
b	67,000,000	−36.2	6,200,000	+6.5
c	85,400,000	−18.7	5,400,000	−7.2
d	99,800,000	−5.0	4,000,000	−31.3
e	95,600,000	−9.0	4,000,000	−31.3
f	78,800,000	−25.0	3,600,000	−38.2
g	142,000,000	+35.2	4,800,000	−17.5
h	105,000,000	0.0	6,800,000	+16.8
i	184,000,000	+75.2	5,600,000	−3.8
j	144,000,000	+37.1	6,000,000	+3.1
Averages	105,000,000		5,820,000	

*The dilutions for Sample a′, Table 15, were prepared at the same time as those for Sample a; those for Sample b′, Table 15, at the same time as those for Sample b, etc.

TABLE 17.—NUMBER OF *B. subtilis* FOUND IN THREE SAMPLES OF FRESH PULP TO WHICH THIS ORGANISM HAD BEEN ADDED IN THE RATIO OF 8:4:1.

SAMPLE	PLATE METHOD		LIQUID MOUNT		STAINED MOUNT	
	Number per cc.	Ratio	Number per cc.	Ratio	No. per cc.	Ratio
As	1,860,000	2.94	132,000,000	5.50	22,600,000	11.41
Bs	1,390,000	2.20	84,600,000	3.94	9,160,000	4.63
Cs	632,000	1.00	24,600,000	1.00	1,980,000	1.00

Series 2.—In the second series in which *Bact. coli* was added to fresh pulp, the methods employed were similar to those used in the preceding series. In addition to the examination of the fresh material the uninoculated pulp and the three inoculated samples were boiled and concentrated one-half, placed in sterile vials, sealed, and later examined by the liquid-mount method and by the dried-and-stained-mount method. The three samples of fresh material were designated as Samples Ac, Bc, and Cc, respectively, while the processed samples were designated as Samples Acx, Bcx, and Ccx. Tables 18 to 26, inclusive, show the results obtained.

Before determining the ratios of *Bact. coli* obtained in Samples Ac, Bc, and Cc, the number of rod-shaped bacteria in the fresh uninoculated pulp, as determined by each method (Tables 18 and 19), was deducted from the total number of rod-shaped bacteria obtained

from the inoculated pulp by the same method (Tables 20 to 25). The estimated numbers of *Bact. coli* and the ratios between the counts are shown in Table 26.

TABLE 18.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH, HIGH GRADE PULP.

SAMPLE	NUMBER OF COLONIES ON PLATES, DILUTION 1:10,000			COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	20	32	12	213,000	−85.4
b′	38	spreader	spreader	380,000	−74.0
c′	44	14	44	340,000	−76.7
d′	170	194	spreader	1,820,000	+24.6
e′	36	34	28	327,000	−77.6
f′	Agar failed to harden				
g′	Agar failed to harden				
h′	560	370	spreader	4,650,000	+218.5
i′	340	440	spreader	3,900,000	+167.1
j′	16	8	spreader	60,000	−95.9
Average				1,460,000	

TABLE 19.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF THE FRESH PULP.

SAMPLE*	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage of variation from average	Count per cc. of rod-shaped bacteria	Percentage of variation from average
a	28,800,000	−4.6	2,400,000	−52.0
b	24,000,000	−20.5	3,200,000	−36.0
c	28,800,000	−4.6	1,600,000	−68.0
d	32,400,000	+7.3	4,800,000	−4.0
e	30,800,000	+2.0	3,200,000	−35.0
f	30,400,000	+0.7	6,000,000	+20.0
g	29,200,000	−3.3	2,400,000	−52.0
h	29,200,000	−3.3	14,400,000	+188.0
i	32,400,000	+7.3	8,800,000	+76.0
j	36,000,000	+19.2	3,200,000	−36.0
Average	30,200,000		5,000,000	

*The dilutions for Sample a′, Table 18, were prepared at the same time as those for Sample a; those for Sample b′, Table 18, at the same time as those for Sample b, etc.

TABLE 20.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE Ac).

SAMPLE	NUMBER OF COLONIES ON PLATES			COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	Too many to count				
b′	†1120	920	1220	543,000,000	+22.9
c′	† 940	960	780	447,000,000	+1.1
d′	†1560	860	640	510,000,000	+15.4
e′	† 500	2000	860	560,000,000	+26.7
f′	Agar failed to harden				
g′	Agar failed to harden				
h′	* 100	250	spreader	175,000,000	-60.3
i′	* 420	740	800	653,000,000	+47.9
j′	* 120	280	220	207,000,000	-53.2
Average				442,000,000	

†Dilution 1:500,000.
*Dilution 1:1,000,000.

TABLE 21.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF FRESH PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE Ac).

SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	93,600,000	+3.2	49,600,000	+40.1
b	78,000,000	-14.0	36,000,000	+1.7
c	96,000,000	+5.8	27,200,000	-23.2
d	96,800,000	+6.7	29,200,000	-17.5
e	83,200,000	-8.3	25,600,000	-27.7
f	80,000,000	-11.8	41,600,000	+17.5
g	87,200,000	-3.9	41,200,000	+16.4
h	81,600,000	-10.0	45,200,000	+27.7
i	111,000,000	+22.4	23,600,000	-33.3
j	100,000,000	+10.3	35,200,000	-0.3
Averages	90,700,000		35,400,000	

*The dilutions for Sample a′, Table 20, were prepared at the same time as those for Sample a; those for Sample b′, Table 20, at the same time as those for Sample b, etc.

TABLE 22.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE Bc).

SAMPLE	NUMBER OF COLONIES ON PLATES			COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	†260	260	230	2,500,000	-98.2
b′	†640	810	660	7,030,000	-95.0
c′	†920	800	1,380	10,300,000	-92.7
d′	*230	240	240	118,000,000	-16.3
e′	*550	560	640	292,000,000	+107.1
f′	Agar failed to harden				
g′	*330	350	340	170,000,000	+20.5
h′	*610	220	200	172,000,000	+22.0
i′	*670	620	660	325,000,000	+130.0
j′	*390	380	280	175,000,000	+24.1
Average				141,000,000	

†Dilution 1:10,000
*Dilution 1:500,000

TABLE 23.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF FRESH PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE Bc).

*SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	68,800,000	-7.9	15,200,000	-11.6
b	77,600,000	+3.9	13,600,000	-20.9
c	88,000,000	+17.8	8,000,000	-53.5
d	97,200,000	+30.1	13,200,000	-23.3
e	72,000,000	-3.6	14,400,000	-16.3
f	68,400,000	-8.4	30,400,000	+76.7
g	60,400,000	-19.2	20,400,000	+18.6
h	66,800,000	-10.6	16,800,000	-2.3
i	71,600,000	-4.2	24,400,000	+41.8
j	76,400,000	+2.3	16,000,000	-7.0
Averages	74,700,000		17,200,000	

*The dilutions for Sample a′, Table 22, were prepared at the same time as those for Sample a; those for Sample b′, Table 22, at the same time as those for Sample b, etc.

TABLE 24.—AGAR PLATE COUNTS OBTAINED FROM DUPLICATE SAMPLES OF FRESH PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE Cc).

SAMPLE	NUMBER OF COLONIES ON PLATES, DILUTION 1:10,000			COUNT PER CC.	PERCENTAGE VARIATION FROM AVERAGE
	Plate 1	Plate 2	Plate 3		
a′	170	170	150	1,630,000	-27.9
b′	180	150	230	1,870,000	-17.3
c′	100	290	220	2,030,000	-10.1
d′	110	120	spreader	1,150,000	-49.1
e′	152	80	spreader	1,160,000	-48.7
f′	80	80	70	767,000	-66.1
g′	124	280	spreader	2,020,000	-10.6
h′	660	880	spreader	7,700,000	+240.7
i′	280	390	280	3,170,000	+40.3
j′	80	50	190	1,070,000	-52.7
Average				2,260,000	

TABLE 25.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF FRESH PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE Cc).

SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
a	51,600,000	+3.4	10,800,000	+28.0
b	44,000,000	-11.8	7,600,000	-10.0
c	44,400,000	-11.1	6,000,000	-28.0
d	49,600,000	-0.6	5,600,000	-33.7
e	51,200,000	+2.6	10,400,000	+23.2
f	44,000,000	-11.8	7,200,000	-14.7
g	47,600,000	-4.6	12,000,000	+42.2
h	54,400,000	+9.0	11,200,000	+32.7
i	56,800,000	+13.8	10,000,000	+18.5
j	55,600,000	+11.4	3,600,000	-57.4
Averages	49,900,000		8,440,000	

*The dilutions for Sample a′, Table 24, were prepared at the same time as those for Sample a; those for Sample b′, Table 24, at the same time as those for Sample b, etc.

TABLE 26.—NUMBER OF *Bact. coli* FOUND IN THREE SAMPLES OF FRESH PULP TO WHICH THIS ORGANISM HAD BEEN ADDED IN THE RATIO OF 8:4:1.

SAMPLE	PLATE METHOD		LIQUID MOUNT		STAINED MOUNT	
	Number per cc.	Ratio	No. per cc.	Ratio	No. per cc.	Ratio
Ac	441,000,000	551.0	60,500,000	3.07	30,400,000	8.84
Bc	140,000,000	175.0	44,500,000	2.26	12,200,000	3.55
Cc	800,000	1.0	19,700,000	1.00	3,440,000	1.00

Upon completion of the analysis of the uninoculated pulp and of the samples to which *Bact. coli* had been added, a period of five days, the surplus from each was boiled for about one-half hour in an open beaker until the concentration had reached approximately one-half of the original amount. Each sample was sealed in a glass vial with a metal cap. Several weeks later these samples were examined for rod-shaped bacteria by both the liquid-mount and by the dried-and-stained-mount methods. The details of the dilution were the same as those employed in the examination, except that the material was diluted 16 times so as to make it of the same consistency as in the first analysis. Ten separate dilutions were made of the original pulp and of each inoculated sample. Tables 27 to 31, inclusive, show the results obtained.

Before determining the ratios between the *Bact. coli* counts obtained in Samples Acx, Bcx, and Ccx, the estimated number of rod-shaped bacteria in the uninoculated processed pulp, as determined by each method (Table 27), was deducted from the total number obtained in each inoculated sample by the same method (Tables 28 to 30). The estimated numbers of *Bact. coli* and the ratios are recorded in Table 31.

TABLE 27.—MICROSCOFIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF THE CONCENTRATED PROCESSED PULP. Compare Table 19.

SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage of variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
xa	4,800,000	−34.8	12,800,000	+77.8
xb	9,600,000	+30.4	28,800,000	+300.0
xc	5,600,000	−22.6	2,400,000	−66.7
xd	4,000,000	−45.7	800,000	−88.9
xe	8,000,000	+8.7	3,200,000	−55.6
xf	4,800,000	−34.8	800,000	−88.9
xg	8,800,000	+19.7	2,400,000	−66.7
xh	6,400,000	−13.0	800,000	−88.9
xi	7,200,000	−0.7	10,400,000	+44.4
xj	14,400,000	+97.0	9,600,000	+33.3
Averages	7,360,000		7,200,000	

TABLE 28—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF THE CONCENTRATED PROCESSED PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE ACX).
Compare Table 21.

SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage of variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
xa	31,200,000	+7.6	19,200,000	-44.7
xb	36,000,000	+24.1	32,800,000	-3.5
xc	35,200,000	+21.4	36,800,000	+8.2
xd	27,200,000	-6.2	36,000,000	+5.9
xe	37,600,000	+29.7	20,000,000	-41.2
xf	24,000,000	-17.2	45,600,000	+34.1
xg	24,000,000	-17.2	32,800,000	-3.5
xh	21,600,000	-25.5	34,400,000	+1.2
xi	25,600,000	-11.8	43,200,000	+27.1
xj	27,200,000	-6.2	39,200,000	+15.3
Averages	29,000,000		34,000,000	

TABLE 29.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF THE CONCENTRATED PROCESSED PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE BCX).
Compare Table 23.

SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
xa	11,200,000	-37.1	20,000,000	-4.8
xb	12,000,000	-32.6	17,600,000	-16.2
xc	16,000,000	-10.1	20,000,000	-4.8
xd	26,400,000	+48.3	27,200,000	+29.5
xe	24,000,000	+34.8	54,400,000	+159.0
xf	20,800,000	+16.8	20,800,000	-1.0
xg	19,200,000	+7.9	10,400,000	-50.5
xh	13,600,000	-23.6	12,800,000	-39.0
xi	20,000,000	+12.4	14,400,000	-31.4
xj	15,200,000	-14.6	12,800,000	-39.0
Averages	17,800,000		21,000,000	

TABLE 30.—MICROSCOPIC COUNTS OBTAINED FROM LIQUID MOUNTS AND FROM DRIED AND STAINED MOUNTS OF THE CONCENTRATED PROCESSED PULP TO WHICH *Bact. coli* HAD BEEN ADDED. (SAMPLE CCX).
Compare Table 25.

SAMPLE	LIQUID MOUNT		STAINED MOUNT	
	Count per cc. of rod-shaped bacteria	Percentage variation from average	Count per cc. of rod-shaped bacteria	Percentage variation from average
xa	17,600,000	+8.0	11,200,000	-11.1
xb	15,200,000	-6.7	8,000,000	-36.5
xc	18,400,000	+12.9	21,600,000	+71.4
xd	10,400,000	-36.2	8,800,000	-30.1
xe	18,400,000	+12.9	5,600,000	-55.6
xf	16,800,000	+3.1	11,200,000	-11.1
xg	19,200,000	+17.8	8,000,000	-36.5
xh	19,200,000	+17.8	8,000,000	-36.5
xi	15,200,000	-6.7	6,400,000	-49.2
xj	12,800,000	-21.7	36,800,000	+192.2
Averages	16,300,000		12,600,000	

TABLE 31.—NUMBER OF *Bact. coli* FOUND IN THREE SAMPLES OF PROCESSED PULP TO WHICH THIS ORGANISM HAD BEEN ADDED IN THE RATIO OF 8:4:1.

SAMPLE	LIQUID MOUNTS		STAINED MOUNTS	
	Number per cc.	Ratio	Number per cc.	Ratio
Acx	21,600,000	2.44	26,800,000	4.96
Bcx	10,400,000	1.16	13,800,000	2.53
Ccx	8,960,000	1.00	5,400,000	1.00

DISCUSSION

In the past certain objections have been raised to the Howard method of examination of tomato products. The objections brought forward by Bitting and Bitting (4), for example, may be grouped under the following heads:

(1) Directions are too indefinite concerning the putting of material into the counting chamber, the amount of shaking to be done in diluting the material, the cleanliness or sterility of utensils used, and whether or not sterile water is to be used in dilutions.

(2) The number of molds, yeasts and spores, and bacteria are reported for different quantities of material.

(3) The differentiation between bacteria and bits of protoplasm or

other debris is considered to be impossible, likewise between yeasts and spores, and contracted protozoa and the refractive bodies found in mold hyphæ.

(4) In counting molds no distinction is made as to whether a small bit or a large mass is in the field.

(5) Micrococci are not counted, altho it is not always possible to differentiate between short rods and cocci in unstained specimens. Decomposition may depend more upon the cocci than upon the rods.

(6) The concentration or amount of solid material present is not taken into account.

(7) The length of time which elapses after manufacture until analysis is made is not taken account of, altho this is regarded as an element of importance.

The above objections are also urged by Schneider (5). Vincent (6) calls attention to the importance of counting the micrococci on the ground that they "probably take just as active a part in the deterioration of tomato products as do the bacilli." Duckwall (7) calls attention to the indefiniteness of the method in certain particulars. He objects to the shaking of the material to be examined because the shaking breaks the masses of mold hyphæ into smaller fragments and thus increases the percentage of fields showing molds. Bertarelli and Marchelli (8) call attention to the fact that the Howard method is subject to considerable error. Bitting (2) emphasizes the importance of knowing what micro-organisms produce objectionable changes in the tomato products.

These objections are not supported by comparative data in the publications cited and in fact very little comparative work seems to have been done to determine the importance of the objections raised. About the only published records, other than those from the Bureau of Chemistry, are given by Bigelow (9) as referee for the Association of Official Agricultural Chemists. His work consisted in sending duplicates of eight samples of tomato products to each of 38 laboratories where analysis was made by the Howard method as carried on in these various laboratories. The results of these analyses show a wide range of variation. In one sample the records of the mold examination varied from 4 per cent of the microscopic fields, as determined by one analyst, to 100 per cent of the fields as found by another. The number of yeasts and spores in one sample as determined by different analysts varied from 0 to 850 per 1/60 cmm., and the bacterial count varied from 6 million to 168 million per cc. Bigelow con-

cludes as a result of these comparative analyses that, "the majority of analysts who profess to use the Howard method are not familiar with it." He suggests the necessity of the analyst receiving personal instructions of from two to four weeks from one accustomed to use the method, and the necessity of constant practice to retain a working knowledge of it. He also says that the method is not one that all can learn to use, but that much depends upon the aptitude of the analyst.

A second feature of the work of Bigelow consisted in preparing a series of uniform samples and sending them to 16 selected analysts who had shown a reasonably good working knowledge of the method. The results of these tests will be considered later in this paper (pages 36, 37, and 39). In addition to determining the agreement in results obtained by analysts working with the duplicate samples, data were obtained to ascertain the relation of the concentration of pulp to the number of micro-organisms present. This was accomplished by taking the samples at intervals from the same kettle at varying degrees of concentration. Table 32, page 45, shows the results as reported by Bigelow (9).

ACCURACY OF HOWARD METHOD IN DETERMINING NUMBER OF MICRO-ORGANISMS PRESENT

In determining the accuracy of the Howard method in the present investigation, two other methods were used for comparison—the agar plate method and the dried-and-stained-mount method—both of which have been in use for several years in estimating the number of micro-organisms in milk and other substances and have been recognized officially by the American Public Health Association as standard procedures.

Molds.—In seeking to determine the accuracy of the Howard method in estimating the amount of mold present, no attempt was made to secure a count by the plate method. Because of Howard's method of reporting the mold content, no exact check on the method could be used. A comparison of the number of fields showing mold hyphæ in the fresh pulp, processed pulp, and catsup in the same sample with the number of hyphal fragments present in the material is the only way in which to check the accuracy of the results secured by this method. This comparison is not satisfactory, owing to the counting of areas, or microscopic fields, in the one case and of hyphal fragments in the other. In this comparison those samples in which mold hyphæ were

present in 100 per cent of the fields are disregarded as increases in the amount of mold present can not increase the percentage over 100. The percentage variation of the results is shown in Table 2, the fresh pulp in each case being reckoned as 100 per cent.

As the processed pulp was not concentrated, the amount of mold in a given volume should be the same in the processed pulp as in the fresh pulp. In the catsup approximately twice as much should be present as in the fresh pulp, in view of the concentration in making the catsup. It is evident from Table 2 that there is considerable variation in the results from the individual samples as determined by the liquid mount, a result substantially in accord with that secured by Bigelow. Wide variations in results also appear in the counts from stained mounts.

If the actual counts (not the percentages as given in Table 2) from each of these samples are added (Table 1) and the average taken, the percentage variations computed from these averages are as follows: By the Howard method in fresh pulp 100 per cent, in processed pulp 103 per cent, and in catsup 154 per cent. By the dried-and-stained-mount method, in fresh pulp 100 per cent, in processed pulp 102 per cent, and in catsup 91 per cent. This comparison shows that the average count from the processed pulp by both methods was nearly the same as that from the fresh pulp. The individual results from the catsup examination show a much wider range of variation than those from the processed pulp, and even the average figures fail to show the doubling in amount that should occur.

In the last two columns of Table 1 are shown the results obtained from samples of catsup when a determination was made of the number of fragments per cc. by the liquid mount and by the dried-and-stained-mount methods. Altho there is a considerable difference in the results secured by the two methods in the case of Samples 1b and 14b, nevertheless if the average counts for the 14 samples are compared the difference is only about 3 per cent. This suggests a means of making mold examinations that gives a more nearly accurate picture of the true conditions than is secured from results based on the percentage of fields showing mold hyphæ.

One of the main objections to the Howard method as applied to estimating the amount of mold present appears to be reporting the presence or absence of mold hyphæ in a given number of fields of the microscope, disregarding the amount of mold in each field. Considerable differences of findings may result from variations in the amount

of shaking given the sample by different analysts as well as variations in the spreading of the sample on the slide. Such variations would be reduced if the number of hyphal fragments in a given amount of the solution were counted, rather than the number of microscopic fields. The kind of fungus is also important, for in some cases the hyphæ break apart much more readily than in others.

In the comparative work reported by Bigelow, the results of the mold examinations from duplicate samples were extremely variable as shown by different analysts. Thus in Series 2, where the average percentage of fields containing molds was 17, the variation was from 4 to 36 as determined by different analysts. In Series 3, with an average of 53, one analyst reported 6 and two others reported 66 per cent of the fields to contain mold. In Series 4, with an average of 64 per cent of the fields containing mold, the reports varied from 22 to 78. With so much variation in the findings of molds in the same sample by analysts selected because of their skill with the method and also with a very large variation in the results where the analyses are made by a single analyst from duplicate samples, the conclusion seems warranted that the Howard method for determining the molds in tomato products cannot be depended upon to give very accurate results.

Yeasts and spores.—Table 3 shows the number of yeasts and spores present in samples of fresh pulp, processed pulp, and catsup as estimated by the liquid-mount and by the dried-and-stained-mount methods. In order to compare the results obtained by the two methods, the percentage variations are given in Table 4. There is a marked variation in the results from some samples and this variation is most noticeable in those samples having a low count, as would be expected. The total counts of yeasts and spores for the 21 samples in which the count was determined in fresh pulp, processed pulp, and catsup by each of the two methods are as follows (Table 3): In the liquid mount the total count from the fresh pulp is 30,500,000 (100 per cent); for the processed pulp, 50,500,000 (166 per cent); and for catsup by Method A, 72,100,000 (236 per cent) and by Method B, 82,800,000 (271 per cent). In the dried and stained mount method the total counts are for fresh pulp 75,500,000 (100 per cent), for processed pulp 47,100,000 (62 per cent), and for catsup 64,300,000 (85 per cent). The processed pulp was of the same consistency as the fresh pulp, the difference between the two being that the processed pulp was subjected to running steam for one hour and stood in sealed

tubes for several weeks. The catsup differed from the processed pulp in that spices, salt, sugar, and vinegar were added, and the volume reduced to approximately one-half by boiling.

The difference in number of yeasts and spores in the fresh pulp as determined by the two methods is large, two and one-half times as many being found by the stained-mount method as by the liquid-mount method. In the processed pulp the total counts as determined by the two methods are more nearly the same, i.e., 50,500,000 and 47,100,000. As determined by the liquid-mount method, this number of yeasts and spores represents an increase of about 66 per cent of the number found in the fresh pulp, while by the dried-and-stained-mount method it represents a decrease of about 48 per cent. In the catsup an increase in the number of approximately 100 per cent would be expected because of the concentration. As determined by the liquid-mount method, there is an increase of 136 per cent in the catsup as compared with the fresh pulp, and of 43 per cent over the processed pulp. With the dried-and-stained-mount method there is a decrease in the catsup of 15 per cent from the fresh pulp and an increase of 36 per cent over the processed pulp.

In the yeast and spore count as reported by Bigelow the greatest variation on a single sample, as shown in Series 3, was from 6, as reported by one analyst, to 1,000 in 1/60 cmm., as reported by another. In Series 1 one analyst reported 3 and another 235; while in Series 2 the extremes were 3 and 105; in Series 4, 7 and 400; in Series 5 and 6, 4 and 600; in Series 7, 4 and 480; and in Series 8, 3 and 450. These figures represent the extreme variation in results in each series of samples and indicate, in a general way, the variations due to the method employed. From these data, as well as from an examination of Table 4, one is led to conclude that there is a large error in the results as obtained by either method the exact cause of which is not entirely clear.

Some light regarding the sources of the errors may be gained by comparing the counts made on catsup by the liquid mount method as shown in Columns 7 and 8, Table 3. These counts were made from the same material. In Method A, only 1/20 cmm. was examined, whereas in Method B ½ cmm. was examined. Ten different mounts from five different dilutions were made, 1/20 cmm. being counted in each mount. The results recorded in Column 7 (A) were computed from the first mount, while the results given in Column 8 (B) are the average of the counts made from the five separate mounts. Taking

the estimate for all of the samples there is a difference in the results of about 15 per cent between the two methods.

By an examination of Column 9, Table 3, it becomes apparent that the percentage variations in results from the samples taken from the same material extend from 3.2 per cent to 330 per cent. Since all of the mounts in any one case were made in a similar way and were taken from the same diluted material, this large variation in results is probably largely due to the examination of too small a quantity of material to secure a fair average in the case of the mount where only 1/20 cmm. was examined.

In considering the sources of error, in the counts from the dried and stained mounts there are at least the following possibilities: Errors may arise from the method of measuring the material tobe examined and of putting it on the slide, from counting too small an amount of material, from the failure of some of the organisms to adhere to the slide, or from the failure of the organisms to stain after being heated. The importance of this latter source of error is shown by a decrease in numbers found after processing in all but 3 of the 21 samples examined (Table 3).

Rod-shaped bacteria.—In considering the accuracy of the Howard method as a means of estimating the number of rod-shaped bacteria attention may be directed to Tables 5 and 6 which show the estimates made from fresh pulp, processed pulp, and catsup by the liquid-mount and by the dried-and-stained-mount methods. Considering those cases in which the three samples of each material were examined and taking the sum of the estimated numbers, the liquid-mount method in fresh pulp gave 1,100,000,000 (100 per cent); in processed pulp, 1,410,000,000 (128 per cent); and in catsup by Method A, 2,440,000,000 (222 per cent) and by Method B, 2,500,000,000 (227 per cent). The dried-and-stained-mount method in fresh pulp gave 3,330,000,000 (100 per cent); in processed pulp, 1,420,000,000 (43 per cent); and in catsup, 827,000,000 (25 per cent). The most conspicuous feature shown by these estimates is the reduction in numbers as determined by the dried-and-stained-mount method in the processed pulp and in the catsup as compared with the numbers from the fresh pulp. This reduction holds true not only for the total found in all samples, but also for the results obtained from each sample with a single exception. This reduction in count due to the processing is similar to the effect noted just above on the yeast and spore counts. It is probable that heat destroys the power of bacteria to absorb the methylene

blue stain. This conclusion seems to be warranted by the fact that the number of rod-shaped bacteria in the processed pulp and catsup, as determined by the liquid-mount method, is nearly as large as was expected. From these data it appears that the liquid-mount method gives more nearly accurate results for comparing processed pulp and catsup with fresh pulp than does the dried-and-stained-mount method.

In estimating the number of rod-shaped bacteria in catsup by the liquid-mount method, the only difference in Methods A and B (Columns 7 and 8, Table 5) consisted in counting the organisms in a greater amount of material in B than in A. In Method A 5/800 cmm. was counted and in Method B 16/800 cmm. In the total numbers in the two cases there is a difference of only about 2 per cent. Column 9 shows the percentage variations in the results of individual samples.

It may be noted that there is a percentage variation in the results from individual samples of from 1.4 per cent to 55.3 per cent. This variation is probably chiefly due to the small amount of material examined and naturally must affect the accuracy of the counts in Method A. It appears, therefore, that the small amount of material that is actually examined causes large and variable errors. In comparing this estimate with that made by Methods A and B for yeasts and spores (Tables 3 and 4) it may be noted that ten separate mounts were used in securing the yeast and spore counts, whereas the counts of rod-shaped bacteria were all made from the same mount. The variation in results from individual samples in the yeast and spore estimate was from 3.2 per cent to 330 per cent, while the variations in the estimates of the rod-shaped bacteria were from 1.4 per cent to 33.3 per cent. Altho the number of rod-shaped bacteria in a given amount of material is greater and the amount of material examined in the two cases is different, yet the evidence indicates that the greater variation in the case of the yeast and spore count is largely due to the difficulty in making satisfactory duplicate mounts.

Vincent (6) finds considerable variation in the results from the Howard method as compared with those from the dried-and-stained-mount method, the variations amounting to as much as 300 per cent in one case. She also finds marked differences in duplicate counts made by either method, the variation being somewhat greater in the case of the liquid mounts.

In the bacterial counts from duplicate samples given in Bigelow's paper Series 1 shows that one analyst reported 3 million per cc. and

another 27 million, the average being about 10 million. In Series 2 the extremes were 4 and 46 million; in Series 3, 3 and 45 million; in Series 7, 110 and 1100 million; and in Series 8, 350 and 2477 million.

Inoculation with a pure culture of a rod-shaped bacterium.—Another phase of the present investigation which has a direct bearing upon the accuracy of the Howard method as a means of estimating the number of rod-shaped bacteria is that in which a broth culture of a known rod-shaped bacterium was added to tomato pulp in different amounts and the recovery of the rods by different methods attempted. Table 17 is a summary of the results obtained when a culture of *B.subtilis* was used. It will be observed in the table that the ratio between the counts from Samples As, Bs, and Cs, which should have been 8 : 4 : 1, was actually 2.94 : 2.20 : 1.00, as determined by the plate method, 5.50 : 3.94 : 1.00 by the liquid-mount method, and 11.41 : 4.63 : 1.00 by the dried-and-stained-mount method.

Table 26 summarizes the results obtained when different proportions of a broth culture of *Bact. coli* were added to different samples of tomato pulp. In this case the expected ratio was 8 : 4 : 1; but the ratios obtained by the plate method were 551 : 175 : 1, by the liquid-mount method 3.07 : 2.26 : 1.00, and by the dried-and-stained-mount method 8.84 : 3.55 : 1.00.

In the work with *B. subtilis* the ratios obtained are rather wide of the mark, indicating a considerable error somewhere in the work or in the methods. One difficulty encountered was the tendency for the organisms to remain in chains, a feature which interfered with an even distribution of the organisms in the solutions. On the plates several colonies were found growing together, apparently from a chain of cells, while in the liquid and stained mounts chains of cells were common. This feature introduced considerable errors into the counts, and so in the ratios, and probably accounts for much of the discrepancy. The results are given, as it is believed that this is the first time that such a check on the accuracy of these counts has been attempted; and even the failure to get results of value is instructive. Because of the unsuitability of the *B. subtilis* culture, further work was done using *Bact. coli.*

The work with *Bact. coli* was more satisfactory, as the organisms were not joined into chains so that the mixing was probably much more uniform. The ratios found in the case of the plate counts are, however, far from the expected, especially the ratio of the average count from Sample Cc to the average counts from Samples

Bc and Ac (Table 26). The ratio of the average count from Sample Ac to that from Sample Bc of 3.15 : 1 approaches more nearly the expected ratio and indicates that for some reason that is not evident a large error has crept into the count from Sample Cc. Comparing the ratios as obtained by the liquid-mount and by the dried-and-stained-mount methods, it will be seen that those obtained by the latter method approach rather closely to the expected ratio; whereas those from the liquid mount method show somewhat greater variation. As the liquid count is larger than that from the stained slides, it appears probable that a large error in the former count has been caused by mistaking cell debris for bacteria, a difficulty that causes much greater trouble in the case of unstained material than in the case of stained material.

Table 31 shows the summary of the counts from the processed samples containing *Bact. coli*. Altho the ratios from the three samples by each method are not very close to the expected ratio of 8 : 4 : 1, the ratio from the results obtained by the dried-and-stained-mount method is closer to the expected than from the results obtained by the liquid-mount method. The ratio between Samples Acx and Bcx is much closer than between Bcx and Ccx, doubtless due to the greater number of organisms present in a given area in the former. It should be noted that in the counts from the fresh pulp with both *B. subtilis* and *Bact. coli* the number found by the liquid-mount method is much higher than in the dried-and-stained-mount method. Since equal volumes were examined in each case and since the material was taken from the same diluted sample, it is probable that the difference is due either to failure to distinguish between debris and bacteria or in the failure of bacteria to stick to the slide in the staining process. The former supposition is the more probable, since in the processed pulp containing *Bact. coli* the number found in the liquid mounts is much less than the number found in the fresh pulp and less than that obtained by the dried-and-stained-mount method.

Tables 9 to 30, inclusive, also show the variations in results from duplicate samples. Tables 9, 11, 13, and 15 show the results obtained by the plate method from fresh, high grade pulp and from the same pulp containing varying proportions of a culture of *B. subtilis*. The variation from the average for each sample in the case of pulp (Table 9) ranges from 51.7 per cent below the average to 101.0 per cent above. These extremes are due in part to the few colonies on each plate and in part to the clusters of colonies. Tables 11, 13, and 15 show

rather wide ranges of variation which, in Sample As (Table 11), seems to be due to an uneven distribution of the organisms in the pulp, since the plates from the same diluted sample do not have such a wide range of variation as do the plates from different diluted samples. This difficulty does not appear to be as great in the case of Samples Bs and Cs in which there were fewer organisms. In these cases the counts secured are reasonably uniform. Another important factor which causes discrepancies in the plate counts is the clumping of the organisms. This has been shown to be an important factor by Conn (10), Breed and Brew (11), and others. On the plates the colonies were frequently clustered, especially the *B. subtilis*-like colonies. In addition to these factors the lack of a uniform consistency in tomato material appears to be another important element causing variations in the counts from plates made from different dilutions as well as in the case of plates made from the same dilution. On the stained slides tomato cells or fragments with which several bacterial cells were associated appeared quite frequently. It is conceivable that one or several such cells or fragments might be drawn up into a pipette at one time and not at another when plates were being made and thus cause considerable variation in counts from individual plates made from the same dilution. A similar condition would hold when the undiluted material was taken from the flask and put into the dilution bottles.

The results obtained by the liquid-mount and by the dried-and-stained-mount methods for the work with *B. subtilis* are shown in Tables 10, 12, 14, and 16. The range of variation in the counts from duplicate samples is greater in the dried-and-stained-mount method than in the liquid-mount method. This likewise holds true for the work with *Bact. coli* both in fresh and processed material. It appears from this that the liquid-mount method gives more uniform results than the dried-and-stained-mount method, yet the ratios obtained by the latter method in the work with *Bact. coli* approach the expected ratios much more closely than do those obtained by the liquid mount method. This fact indicates that, in spite of the greater variation in the individual samples, the results obtained from the fresh pulp by the dried-and-stained-mount method really show the true number of organisms present more accurately than the results with the liquid-mount method. The counts by one method or the other must be very inaccurate as the counts from the liquid mounts are far in excess of those from the stained mounts. Another feature of some

significance is that the variation from the general average, as determined by the two methods, is not uniform for single diluted samples. For example, Sample b in Table 14 shows a variation of 14.2 per cent from the average by the liquid-mount method and 69.3 per cent by the dried-and-stained-mount method. This is a rather extreme example but it illustrates the general condition found in the work both with *B. subtilis* and with *Bact. coli* in both fresh and processed pulp. From this it would seem that the variation was not caused so much by variations in preparing the dilutions as in the details of mounting and counting. Possibly the capillary pipette used in putting the material on the slide in the dried-and-stained-mount method is an important factor in causing this variation.

Total bacteria.—Another phase of the present investigation which has a bearing on the general subject under consideration is the estimate of the total number of bacteria, including coccus forms as well as rods. Tables 7 and 8 give the results of counts of this type. Taking the sum of the number obtained from 21 of the samples, the liquid-mount method shows 2,370,000,000 (100 per cent) in fresh pulp, 2,420,000,000 (102.1 per cent) in processed pulp, and 4,030,000,000 (170.0 per cent) in catsup. The dried-and-stained-mount method shows 4,150,000,000 (100 per cent) in fresh pulp, 1,720,000,000 (41.4 per cent) in processed pulp, and 941,000,000 (22.7 per cent) in catsup. The plate count showed 2,200,000,000 colonies or 92.8 per cent of the number shown by the liquid mount on fresh pulp. These results harmonize with those obtained from counting the rods only, and confirm the conclusions drawn from these data.

General discussion.—The range of variation or the probable error of the method of determining the number of organisms present in a given amount of material can hardly be computed with any degree of accuracy from these results because of the small amount of data available, yet a few features may be noted. In determining the amount of mold present it may be observed by referring to Table 1 that the variation, as determined by the Howard method, between the fresh pulp and the processed pulp of the same sample in which the counts should be the same is, in Sample 1a, 6 per cent of the microscopic fields in fresh pulp and 30 per cent in processed pulp, a variation of 400 per cent. In Sample 11b, on the other hand, there were 52 per cent of the microscopic fields showing mold in fresh pulp and 46 per cent in processed pulp, a variation of 11.5 per cent. In general, the greatest variation occurs in those samples showing the lowest

counts. Altho not entirely comparable because the work was done by different analysts, Bigelow gives an analysis of duplicate samples for molds in which one analyst reported 6 per cent and two others 66 per cent of the fields as showing mold hyphæ. In another case, a duplicate sample of the material was sent to the same analyst without his knowledge and he reported 60 per cent of the fields to show molds for one sample and 26 for the other, while the average for all analysts was 54. It would seem, therefore, from the data available that the results of the mold examination by the Howard method as used give only a very general idea of the amount of mold present in a given sample.

As for yeasts and spores a comparison of the estimated numbers and percentages in the fresh and processed pulp of the same sample as given in Tables 3 and 4 show as great variations as do the estimates of the amount of mold present. Also the two estimates on the same sample of catsup show variations as large as 330 per cent in Sample 9b (Column 9, Table 3). Bigelow mentions one case where one analyst reported 1,000 yeasts and spores per 1/60 cmm., while another analyst reported 6 from a duplicate sample. On duplicate material from the same sample, a single analyst reported 8 yeasts and spores per 1/60 cmm. at one time and 16 at another.

In estimating the number of rod-shaped bacteria by the Howard method the results as shown in Tables 5 and 6, as well as the results reported by Bigelow, point to the same general conclusion as that drawn from a study of the results from the mold examination and from the count of yeast cells and spores, namely, that the method as used cannot be depended upon to give more than a general idea of the number of micro-organisms present in a given sample.

EFFECT OF CONCENTRATION UPON NUMBER OF MICRO-ORGANISMS PRESENT

In the present investigation concentration was employed in the making of catsup from the processed pulp, the reduction in total volume approximating 50 per cent in each case. The amount of total solids was determined in several samples, both on processed pulp and on catsup. The average on processed pulp for 10 samples was 4.62 per cent, and in catsup, 17.49 per cent for 18 samples. The addition of spices, salt, and vinegar would account, in part at least, for the discrepancy between the reduction of volume and the percentage of solids found. An examination of Table 1 shows that in

the catsup there is an increase in several samples in the number of hyphal fragments above that found in the processed pulp, as well as in the fresh pulp. If the estimated numbers from the 21 samples in which catsup was made are added together, the percentages of hyphal fragments are 100 in fresh pulp, 118 in processed pulp, and 263 in catsup. This clearly shows an increase in count due to concentration. In the Howard method, considering only those samples which show less than 100 per cent of fields (Table 2) and taking an average,

TABLE 32.—EFFECT OF CONCENTRATION OF TOMATO PULP ON THE NUMBER OF MICRO-ORGANISMS, AS RECORDED BY BIGELOW.

SERIES NUMBER	PERCENTAGE OF SOLIDS	MOLDS IN PERCENTAGE OF FIELDS, AVERAGE	YEASTS AND SPORES PER 1/60 CMM., AVERAGE	MILLIONS OF ROD-SHAPED BACTERIA PER CC., AVERAGE
1	4.96	27	7	13
	5.88	25	8	10
	7.34	30	8	12
	10.58	34	11	17
2	5.00	13	5	8
	8.58	17	8	10
	15.22	22	10	20
3	5.03	53	13	13
	6.12	52	15	10
	8.81	51	15	12
	13.17	54	27	19
4	5.47	60	15	18
	6.35	64	12	14
	8.39	65	14	14
	9.70	66	22	18
5	5.33	17	6	81
	12.98	13	10	279
6	17.20	12	12	598
	22.60	20	11	714
7	5.00	16	9	416
	8.42	12	13	701
	14.07	12	13	1407
	17.83	14	7	1747
	21.04	12	9	2130
8	4.88	14	16	935
	11.70	15	16	1298
	20.10	14	15	2170
	28.80	13	13	3066

the results in percentages are in fresh pulp 100, in processed pulp 103, and in catsup 154.

Bigelow concludes in his report that "in the concentration on pulp the mold count is not increased." Table 32 shows his general results. There is some increase in molds upon concentration in Series 1, 2, and 6; in Series 3, 4, and 8 there is virtually no change; while in Series 5 and 7 there is a decrease in number, even tho in Series 7 the concentration is over four times. In examining Bigelow's tables it is evident that the difference in number associated with concentration is not as great as the variation in the results secured by the different analysts.

Howard and Stephenson (1) in considering the effects of concentration upon the mold content say that the mold content in sauces does not seem to be much higher than that in the pulp. The data presented in this investigation upon stained hyphal fragments suggest that there is a relation between the number of such fragments and the degree of concentration. If the total number of hyphal fragments of the 21 samples of the processed pulp examined be compared with the total number found in the catsup (Columns 7 and 8, Table 1), the percentages will be 100 for processed pulp and 220 for catsup. Such a relationship should hold true for any method that really gives a fair idea of the amount of mold present.

The results of the present work show an increase in the yeast and spore count due to concentration. This will be seen by examining the sum of the estimates for the 21 samples as determined by the liquid mount method in Table 3 where fresh pulp shows 100 per cent, processed pulp 166 per cent, and catsup 240 per cent. Bigelow records some increase in number upon concentration in some of his series, e.g., Series 1, 2, 3, 4, and 5; but the increase is by no means proportional to the increase in concentration. He makes this comment, "it is unfortunate, however, that all of the samples taken were so low in yeasts and spores that it is entirely possible that the error of the determination would be sufficient to conceal a greater number of those bodies in higher concentration." Howard and Stephenson report that in sauces the increase in the count for yeasts, spores, and bacteria is nearly proportional to the degree of concentration. The estimate made in this work with the dried-and-stained-mount method for yeasts and spores showed in fresh pulp 100 per cent, in processed pulp 62 per cent, and in catsup 85 per cent. The decrease in number

is probably due to a failure on the part of the cells to stain or, possibly, to adhere to the slide.

The number of rod-shaped bacteria increased upon concentration as shown in Tables 5 and 6. As estimated by the liquid mount method, there were 128 per cent in processed pulp and 222 per cent in catsup, as compared to 100 per cent in fresh pulp. Bigelow's tables also show an increase in number with an increase in concentration. He notes that "altho there were a few striking exceptions, the uniformity in general is so great as to suggest that the bacterial count is directly proportional to the concentration of the pulp." This author says further that the lack of increase noted in the first four series with increasing concentration may have been due to the fact that the number of organisms present was no greater than the error of the method.

From all the data available the conclusion seems to be warranted that the liquid-mount method shows the number of fragments of mold hyphæ, yeasts and spores, and rod-shaped bacteria in a given sample of tomato product to be increased when the sample is concentrated, and that the increase in a general way is proportional to the concentration.

Where the dried-and-stained-mount method was used to estimate the number of rod-shaped bacteria (Tables 5 and 6) only 42.5 per cent were found in the processed pulp and 28.9 per cent in the catsup as compared to 100 per cent for the fresh pulp. This reduction in count after processing and even after concentration is similar to the condition noted in the estimate of the number of yeasts and spores. This decrease in number on processing is also shown in the work with *Bact. coli* as reported in the present paper. In this connection the work of Hastings and Davenport (12) is of interest. In working on the effects of pasteurization on the number of bacteria in milk, they found that some bacterial cells lose their staining power while others do not. They also found that when *Bact. coli* is inoculated into milk and then pasteurized there is no apparent change in staining or in the number of organisms present after the process as compared with the number before. Apparently in processing tomato pulp at a temperature of 212° F., a number of these organisms lose their staining property. Winslow and Willcomb (13) also note that there is a gradual and rather rapid disappearance of dead bacterial cells when placed in water, as determined by microscopic counts.

RELATION OF NUMBER OF MICRO-ORGANISMS IN TOMATO PRODUCTS TO AMOUNT OF DECOMPOSITION

It is a generally recognized fact that the decomposition in tomatoes as well as in other organic material is due, in large part at least, to micro-organisms. Bitting, K. G. (4), remarks that "tomato pulp furnishes a medium suitable for the development of many organisms, as it contains all of the necessary food elements. On account of its mild acidity it is especially suitable for the development of many yeasts and molds, and some forms of bacteria; consequently there is present a varied and abundant flora if the pulp be held for an appreciable time before using, or if it has been made from tomatoes not properly sorted and washed." Howard (14) notes that "tomatoes constitute a very suitable medium for a large variety of organisms, and as a result the flora producing decay in this fruit and its products is a varied one." In another connection he makes this statement in speaking of bacteria, "the rod-shaped and spiral form are most easily detected, altho from the standpoint of effect on the product some of the worst forms are of the spherical type."

Two types of spoilage are recognized in tomatoes, *viz.*, "primary spoilage" which takes place before and during the process of manufacture, and "secondary spoilage" which takes place after the product is manufactured and while in storage.

Table 33 shows the percentage of decay in each sample, and the number of organisms obtained by each method in the present investigation and shows the conditions where primary spoilage is present.

From an examination of this table it is evident that there is no uniform increase in the percentage of fields showing mold hyphæ or in the number of hyphal fragments per cc. with an increase in percentage of decay. In fact, the percentage of fields showing mold hyphæ is higher in Sample 13a, with 3.5 per cent of decay, than it is in Sample 27 with 44 per cent. Likewise, in Samples 1a and 10a, each with 8 per cent of decay, one showed 6 per cent of the fields with mold hyphæ and the other 98 per cent.

It is interesting to note that the number of hyphal fragments is the same in Sample 23 from sound stock, in Sample 13b with 4 per cent of decayed stock, and in Sample 10b with 5 per cent of decayed stock. Yet the percentage of fields showing mold hyphæ varies from 0 per cent in the first case to 30 per cent in the second and 22

TABLE 33.—NUMBER OF MICRO-ORGANISMS IN FRESH PULP AS DETERMINED BY DIFFERENT METHODS IN 30 SAMPLES CONTAINING VARYING AMOUNTS OF DECAYED MATERIAL.

SAMPLE NUMBER	PER-CENT-AGE OF ROT	MOLDS		YEASTS AND SPORES		ROD-SHAPED BACTERIA		TOTAL BACTERIA	
		Percentage of fields showing mold hyphae	Hyphal fragments per cc.	Liquid mounts per cc.	Stained mounts per cc.	Liquid mounts per cc.	Stained mounts per cc.	Liquid mounts per cc.	Stained mounts per cc.
2a	0	2	38,200	240,000	535,000	2,160,000	6,650,000	11,300,000	8,480,000
3b	0	0	0	30,000	1,380,000	2,400,000	5,000,000	11,300,000	7,260,000
4a	0	0	0	0	38,200	1,440,000	3,170,000	7,200,000	4,890,000
7	0	0	0	0	306,000	2,880,000	2,560,000	13,900,000	3,210,000
8b	0	0	0	0	153,000	6,000,000	2,410,000	12,700,000	3,170,000
23	0	0	229,000	720,000	6,420,000	13,600,000	15,000,000	70,400,000	28,600,000
13a	3.5	82	840,000	1,560,000	2,330,000	24,700,000	78,900,000	61,200,000	105,000,000
13b	4	30	229,000	420,000	688,000	76,300,000	215,000,000	142,000,000	254,000,000
14a	4	72	191,000	1,860,000	993,000	80,400,000	162,000,000	169,000,000	203,000,000
14b	4	28	38,200	180,000	267,000	51,400,000	93,300,000	106,000,000	113,000,000
11b	4.5	52	420,000	420,000	1,410,000	69,100,000	185,000,000	129,000,000	208,000,000
10b	5	22	229,000	60,000	2,640,000	35,300,000	106,000,000	71,000,000	129,000,000
21	7	16	1,830,000	4,500,000	10,500,000	10,200,000	74,200,000	52,500,000	166,000,000
1a	8	6	76,400	180,000	1,680,000	25,700,000	37,400,000	67,900,000	56,000,000
9b	8	60	344,000	480,000	1,030,000	112,000,000	325,000,000	204,000,000	373,000,000
10a	8	98	2,600,000	5,280,000	3,170,000	67,400,000	171,000,000	144,000,000	228,000,000
11a	9	66	955,000	1,680,000	2,330,000	63,600,000	158,000,000	120,000,000	192,000,000
8a	11	86	1,570,000	4,200,000	8,590,000	87,100,000	140,000,000	220,000,000	216,000,000
1b	16	46	38,200	420,000	382,000	22,100,000	93,500,000	73,200,000	116,000,000
31	17	4	802,000	180,000	3,320,000	14,400,000	27,700,000	40,300,000	37,000,000
30	18	2	0	180,000	3,210,000	22,400,000	54,700,000	59,200,000	70,600,000
24	20	82	115,000	180,000	4,010,000	156,000,000	327,000,000	413,000,000	599,000,000
26	21	96	3,440,000	3,960,000	7,560,000	146,000,000	230,000,000	300,000,000	376,000,000
2b	22	94	1,640,000	4,260,000	23,300,000	90,200,000	393,000,000	231,000,000	538,000,000
3a	30	98	1,800,000	7,200,000	13,400,000	112,000,000	590,000,000	259,000,000	711,000,000
9a	38	96	1,150,000	1,800,000	7,560,000	149,000,000	533,000,000	277,000,000	640,000,000
27	44	32	917,000	3,960,000	15,600,000	234,000,000	149,000,000	614,000,000	239,000,000
19	100	100	13,500,000	34,000,000	7,640,000	57,600,000	78,900,000	240,000,000	311,000,000
20	100	98	917,000	4,320,000	2,750,000	1,300,000,000	2,000,000,000	2,820,000,000	3,750,000,000
25	100	100	11,300,000	20,200,000	20,200,000	136,000,000	499,000,000	243,000,000	887,000,000

per cent in the third, showing that the mold fragments were much more widely distributed in one case than in the other. Howard and Stephenson (1) in their Table 7, show the relation between percentage of rot and microscopic count on laboratory samples. An examination of this table shows marked variations in mold estimates. For example, Sample 21 with 5.5 per cent of decay shows 50 per cent of the fields with molds, while Sample 22 with 7.3 per cent of decay shows no molds. Likewise, Sample 29 with 11.7 per cent decay shows 6 per cent of the fields with mold, while Sample 30 with 12.4 per cent decay shows 54 per cent of the fields with molds. Of course these are extreme examples, but a curve plotted so as to bring out the relationship between decay and fields showing mold hyphæ either in the present investigation or in the report of Howard and Stephenson would be very irregular. Altho sound stock contains relatively few mold hyphæ while bad stock may and usually does contain large numbers of mold hyphæ, yet the conclusion seems unavoidable that by the methods employed in estimating the amount of mold in tomato products there appears to be little definite relationship between the percentage of decayed material in the original stock and the estimated amount of mold.

The estimated number of yeasts and spores likewise bears little relationship to the percentage of decay as determined in the present study either by the liquid-mount or by the dried-and-stained-mount methods, as illustrated by a few extreme examples. Sample 13a with 3.5 per cent decay showed 1,560,000 yeasts and spores by the liquid-mount method and 2,330,000 by the dried-and-stained-mount method, while Sample 1a with 8 per cent of decay showed 180,000 by the liquid mount method and 1,680,000 by the dried-and-stained-mount method. Even perfectly sound stock may show nearly as many yeasts and spores as stock with a large amount of decay. In the work of Howard and Stephenson, Sample 23 with 7.5 per cent of decay showed 240,000 yeasts and spores per cc.; while Sample 29 with 11.7 per cent of decay showed 4,080,000 per cc. and Sample 34 with 19.1 per cent of decay, 540,000 per cc. From the data available the present methods show little if any correlation between the number of yeasts and spores and the amount of decay.

The estimated number of rod-shaped bacteria likewise shows wide variations which do not conform to the percentage of decay. Sample 8a with 11 per cent of decay shows 87 million rods by the liquid-mount method and 140 million by the dried-and-stained-mount

method, while Sample 31 with 17 per cent decay shows only 14 million by the liquid-mount method and 28 million by the dried-and-stained-mount method. Sample 24 with 20 per cent decay shows 156 million and 327 million, respectively, for the two methods. Pulp made from perfectly sound stock was found at times to contain tens of millions of organisms per cc. (Tables 10 and 19), while some pulp, containing as high as 17 per cent of decayed material, showed no more than this. The table of Howard and Stephenson shows similar marked variations as Sample 33 with 18 per cent decay showed 29 million, while Sample 35 with 20 per cent of decay showed 402 million. These are the most conspicuous examples of discrepancies; but, nevertheless, they show so much variation that one is warranted in the conclusion that the estimated number of rod-shaped bacteria as determined by the methods employed does not indicate the percentage of decay in the tomato product. The same conclusion is reached when the total number of bacteria, including rods and coccus forms, is considered.

It may be well to note in this connection that the coccus forms of bacteria as determined in this work do not seem to bear any closer relation to the amount of decay than do the rod-shaped ones.

Howard and Stephenson conclude from their work that, while comparatively low counts may not always indicate a product made from suitable stock, a high count of any one or more of the three types of organisms indicates bad stock. A tomato product is considered by them as unsuitable for human consumption if it shows molds in 66 per cent of the microscopic fields or 125 yeasts and spores in 1/60 cmm. (7,500,000 per cc.), or 100,000,000 rod-shaped bacteria per cc. With these as standards in the present investigation with the fresh tomato pulp, 13 samples (13a, 14a, 10a, 11a, 8a, 24, 26, 2b, 3a, 9a, 19, 20, 25) would have been condemned on the basis of the examination for molds, only 2 samples (19,25) on yeast and spore count, and 8 on the rod-shaped bacteria count (9b, 24, 26, 3a, 9a, 27, 20, 25). (See Table 33). All but two (9b, 27) of the samples which exceeded the limit on bacterial count exceeded the limit in the mold count also.

In the processed pulp the same samples are above the limits in the mold examination as were above in the fresh pulp; while in the yeast and spore count two more are above, and in the bacterial count one more. However, no samples were added to the list as being above the limits in any one of the three counts which was not already above in the mold count in the fresh pulp. In catsup six additional samples were higher than the limit as stated above, two additional

ones (1b, 11b) showing above 66 per cent of the fields with mold hyphæ (Table 1), and three additional ones (31, 1a, 10b) being above 100 million in the count of rod-shaped bacteria (Table 5).

Howard and Stephenson believe that high yeast and spore and bacterial counts usually indicate secondary spoilage, and that the mold count is of greater importance in judging the condition of the raw stock than the counts of the other micro-organisms. The results of the present investigation seem to confirm the latter part of this conclusion.

It is interesting to note that in spite of the very evident inaccuracies in the counts and the lack of correlation between numbers and percentage of decay, all of the samples of catsup containing more than 7 per cent of decayed material failed to meet the standards established by the Bureau of Chemistry. The sample containing the least amount of decayed material which failed to meet the standards was Sample 13a. This contained 3.5 per cent of bad stock. Three samples containing more bad stock than did Sample 13a would have passed the standards set, namely, Samples 13b and 14b with 4 per cent, and Sample 21 with 7 per cent. Yet if the analyst attempted to draw any conclusion regarding the percentage of decayed material in the original stock from the analytical results he would have been badly deceived for Sample 13a, containing only 3.5 per cent of decayed material, showed mold hyphæ more than twice as frequently (82 per cent of the microscopic fields) as did Sample 27 containing 44 per cent of decayed material. Even pulp made from rotten tomatoes did not always show mold hyphæ in every field of the microscope (Sample 20). Some samples containing as much as 17 and 18 per cent of decayed material scarcely showed any mold hyphæ (Samples 31 and 30).

CONCLUSIONS

From the data available, the conclusion seems to be warranted that the Howard method of determining the number of micro-organisms in tomato products can not be relied upon to give an accurate estimate of the number actually present in the material. Without doubt the skill of the analyst plays an important part in securing uniform results as is the case in all analytical work, yet there appear to be more fundamental objections than the "aptitude of the analyst."

Briefly considering the main objections which have been urged

against the method, the following conclusions may be drawn from the present work:

(1) The series of samples used in the present investigation do not indicate that "micrococci" are more abundant in decayed tomatoes than in undecayed ones.

(2) The inability to distinguish between bacteria and bits of protoplasm or other debris appears to cause serious discrepancies in the counts as shown by the work with *B. subtilis* and *Bact. coli.*

(3) The amount of concentration is a factor that should be taken into account. Concentration increases the count wherever the count is based on methods showing the number of hyphal fragments or of micro-organisms present per unit volume.

(4) The fact that the examination of a limited amount of material causes wide errors is one that has not been sufficiently emphasized by previous workers, as it is an important factor in causing discrepancies in the counts. This is shown especially from the results obtained in counting the rod-shaped bacteria and yeasts and spores in different amounts of the same material.

(5) Probably the factor which brings about the greatest amount of variation is the difficulty in making uniform and representative mounts. This is especially apparent from the work with *B. subtilis* and *Bact. coli* in which there was a marked variation in the duplicate counts by both the liquid-mount and by the dried-and-stained-mount methods. A similar variation was shown by the plate count. Howard (15) in a recent paper reports the work of three analysts in using different instruments for putting the material on the mount for the mold count. He found that considerable variation resulted from using the different instruments and concludes that the use of the scalpel gives the most uniform results. It may be noted that a scalpel was used in the present investigation for mounting the material for the mold count.

(6) In view of the marked differences which were found between the results obtained with the three methods when the same sample was examined as well as the marked discrepancies which were found when series of duplicate samples were examined, the conclusion seems justified that the character of the material does not lend itself to the tests ordinarily applied in determining the number of micro-organisms in a given food. The particular characteristics of tomato products which render it more difficult to make these counts accurately appear to be the heterogenous nature of the material and

the adherence of some of the organisms to the particles of tomato suspended in the liquid medium, a condition which was frequently observed in stained material in the present investigation.

In view of the imperfections of the Howard method, admitted even by Howard himself, can any other system of control be used that will give more satisfactory results?

Obviously the agar plate method cannot be used for the determination of the quality of the sterilized pulp and, from present indications, the use of counts from dried and stained mounts of the processed material do not seem to offer any solution of the difficulty, as the number of organisms found in such mounts is much fewer than the number originally present.

Apparently one or the other of two lines of progress are open, *viz.*, (a) the improvement of the liquid mount method for use on the processed material; or (b) the establishment of a control over the quality of the product as made.

The changes in the technic of the microscopic examination suggested by Bartarelli and Marchelli (8) do not appear to offer a real solution of the difficulty, while those of Miller (16) appear to be more promising. He suggests staining the micro-organisms in the liquid materal by the addition of Loeffler's methylene blue and Ziehl-Neilson's carbol-fuchsin. While the author has not tried this modification, it seems to be generally agreed that it is difficult to get satisfactory results with the particular staining procedure suggested. Further study may result, however, in finding a satisfactory staining technic.

Schneider (5), without being very specific, mentions various modifications of the Howard technic commonly used in food laboratories. As a result of the present study, some of these have already been incorporated in the outline of the Howard technic as adopted by the Association of Official Agricultural Chemists[1] and others might well be.

From the present work it appears that the most important suggestions are (a) that every effort be made to secure a uniform and well mixed portion for microscopic examination, (b) that the present system of recording the percentage of fields showing mold hyphæ be changed to one showing the number of hyphal fragments per unit volume, (c) that the magnification used in making the yeast and spore count be the same as that used in making the bacterial counts, and (d) that the amount of material examined in cases where the

[1] *Jour. Assoc. Offic. Agr. Chemists* **6**, *146. 1922.*

accuracy of results is of great importance be made much larger than is specified at present.

But a still more promising plan for developing a satisfactory control of these food products appears to be an inspection of the product as manufactured. From a factory inspection it is possible to determine the quality of the raw stock and this might well be checked by means of laboratory inspection of the fresh pulp. This could probably be made most satisfactorily from dried and stained mounts rather than from liquid mounts. The dried mounts have the advantage not only of furnishing more nearly accurate counts but also of being permanent, so that they could be preserved in those cases where questions concerning the quality of the pulp might be raised later. Such a factory control to be made entirely effective under our laws should be carried out cooperatively by federal and state agencies. The industry itself might also find it to its advantage to establish a similar control over the fresh products in order to maintain the quality desired. A similar control is now being extensively developed by the most progressive of our milk dealers for controlling the quality of milk before pasteurization and is proving very effective in enabling these men to meet the standards of quality set for this food product.

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